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Strains of *Streptococcus mutans* and *Streptococcus sobrinus* Attach to Different Pellicle Receptors

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We compared the levels of adsorption of *Streptococcus mutans* JBP and *Streptococcus sobrinus* 6715 to experimental pellicles formed from unsupplemented and glucosyltransferase (GTF)-supplemented saliva. Pellicles formed on hydroxyapatite beads from GTF or from saliva-GTF mixtures possessed detectable GTF activity. Low levels of GTF activity were also detected in clarified whole human saliva, but not in samples of submandibular saliva. The adsorptive behavior of *S. mutans* JBP to pellicles formed from saliva or saliva-GTF mixtures was strikingly different from that of *S. sobrinus* 6715. *S. mutans* JBP adsorbed in higher numbers to pellicles formed from whole or submandibular saliva than to buffer-treated hydroxyapatite under the assay conditions used, in which blocking with albumin was used. In contrast, *S. sobrinus* 6715 attached in lower numbers and did not show enhanced adsorption to pellicles prepared from saliva. Pellicles prepared from the high-molecular-weight mucin fraction of submandibular saliva effectively promoted adsorption of *S. mutans* JBP, but none of the saliva fractions tested enhanced the attachment of *S. sobrinus* 6715 above the levels of buffer controls. Exposure of pellicles which contained GTF to sucrose to permit in situ synthesis of glucan markedly enhanced attachment of *S. sobrinus* 6715 but not attachment of *S. mutans* JBP. Also, the presence of sucrose throughout the adsorption period did not enhance attachment of *S. mutans* JBP. Both organisms possessed cell-associated GTF, and GTF preparations derived from *S. sobrinus* 6715 and *Streptococcus sanguis* FC-1 behaved like GTF derived from *S. mutans* JBP. *S. sobrinus* 6715 attached in high numbers to dextran-treated hydroxyapatite, whereas *S. mutans* JBP did not. These observations suggest that *S. mutans* JBP cells possess an adhesin which binds to salivary components in the pellicles. In contrast, *S. sobrinus* 6715 cells appear to possess an adhesin which binds to glucan in the pellicles. Four additional strains of *S. mutans* and four additional strains of *S. sobrinus* behaved qualitatively like strains JBP and 6715, respectively, and thus the differences observed appear to be representative of these species. Collectively, our data indicate that *S. mutans* and *S. sobrinus* attach to different receptors in experimental pellicles.

The mutans group of streptococci has been associated with the etiology of dental caries in humans and in experimental animals. Although the members of this group have many phenotypic and pathogenic similarities, they are antigenically (3, 22) and genetically (7, 8) heterogeneous, and the group has been divided into six distinct species. The most common species encountered in humans are *Streptococcus mutans* (formerly *S. mutans* serotypes c, e, and f), which comprises about 90% of the isolates, and *Streptococcus sobrinus* (formerly *S. mutans* serotypes d, g, and h) (16, 21, 27).

Because of their high cariogenic potential, the mode by which these streptococci attach to and subsequently accumulate on teeth has been extensively studied. Early studies suggested that glucan synthesis from sucrose catalyzed by glucosyltransferases (GTF) elaborated by the organisms was required for their attachment to teeth (for reviews, see references 10, 14, and 17). However, subsequent studies showed that strains could be implanted on the teeth of rodents fed sucrose-free diets (33) and that they could attach to experimental salivary pellicles formed on hydroxyapatite (HA) surfaces which were similar to pellicles of teeth in the absence of glucan synthesis (4, 6). Also, studies involving GTF-defective mutants of *S. mutans* or physiologic manipulation of *S. sobrinus* strains have indicated that cell-associated GTF is not essential for and does not enhance the initial attachment of these streptococci to experimental pellicles (5).

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Recently, GTF activity was demonstrated in whole human saliva and in newly formed pellicles (24–26). Because this enzyme and its product, glucan, have been demonstrated to bind to the surfaces of *S. mutans* and other streptococci (15, 18, 20, 30), it has been suggested that the presence of GTF in pellicles plays a role in the attachment of bacteria to teeth (25, 26). In this study we compared the adsorption of *S. mutans* JBP (serotype c) and *S. sobrinus* 6715 (serotype g) to experimental pellicles prepared from unsupplemented saliva and GTF-supplemented saliva. Our data indicate that there are fundamental differences in the natures of the pellicle receptors for these species.

MATERIALS AND METHODS

Cultures and culture conditions. *S. mutans* JBP and MT3 (serotype c) and *S. sobrinus* 6715 (serotype g) were obtained from the culture collection of the Forsyth Dental Center. *S. mutans* S3, P2, and AT6 (serotype c) and *S. sobrinus* OMZ-176, MB2R, 14H, and SL-1 were obtained from Douglas Bratthall. All of the strains were stored in 50% glycerol at −20°C until they were used. For experimental purposes, inocula from the glycerol stock cultures were grown in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.). Cultures were incubated anaerobically in Brewer jars filled with 80% N_2, 10% H_2, and 10% CO_2 at 35°C. Early-stationary-phase cells were used for all assays. For adherence studies the organisms were radiolabeled by growing them in Todd-Hewitt broth supplemented with 10 μCi of [^{3}H]thymidine (New England Nuclear Corp., Boston, Mass.) per ml, as previously described (4, 11).

Preparation of GTF. GTF was prepared by a modification of the affinity procedure of Smith et al. (28). Briefly, the streptococci were grown in a chemically defined medium (29) supplemented with 0.1% Tween 20 and 0.5% glucose. After incubation, the cultures were centrifuged at 10,000 × *g* for 15 min to remove the streptococcal cells, and the culture liquor was adjusted to pH 6.4 by adding 1 N NaOH. The liquor was then filtered through a 0.6-μm membrane filter (Millipore Corp., Bedford, Mass.) to remove residual cells, and 25 ml of packed prewashed hydrated dextran beads (Sephadex G-100; Pharmacia, Uppsala, Sweden) was added per 500 ml of liquor. The mixture was shaken at room temperature for 2 h to permit the enzyme to adsorb to the dextran beads. The beads were then collected, washed once with 0.85% NaCl, and suspended in 25 ml of 6 M guanidine hydrochloride (pH 6.5) for 1 h at room temperature with shaking to elute the GTF. The eluate was collected following centrifugation and dialyzed against several changes of 0.85% NaCl containing 0.04% NaN_3 over a 48-h period. The resulting GTF preparation was stored at 4°C until use. The properties of GTF prepared in this manner have been described previously (28); various preparations contained between 5 and 18 μg of protein per ml.

Analytical methods. Protein was determined by the dye-binding procedure of Bradford (2). GTF activity was assayed by using [^{14}C]glucose-labeled sucrose as described by Germaine et al. (9). The reaction mixtures (200 μl) contained 100 μl of 0.8% sodium acetate buffer (pH 5.5) supplemented with 0.06% NaF and 0.04% dextran 10,000 (Pharmacia), varying quantities of enzyme or saline, and 20 μl of 6.8% sucrose containing 25 μCi of radiolabeled sucrose per ml. All solutions were prewarmed to 35°C prior to mixing. Triplicate 10-μl samples were removed at zero time and at 10, 20, and 30 min after the reaction was initiated. These samples were spotted onto filter paper disks (diameter, 2.4 cm), which were immediately placed into a beaker containing absolute methanol (at least 10 ml per disk) and permitted to stand for at least 15 min. The disks were then removed and placed in fresh methanol for an additional 15 min. This washing procedure was repeated a third time, and then the disks were air-dried and placed in scintillation vials for counting. GTF activity was expressed as micrograms of glucan formed per hour per milliliter of enzyme preparation from [^{14}C]glucose-labeled sucrose. Total polysaccharide synthesis was determined by using uniformly ^{14}C-labeled sucrose. The difference between total polysaccharide synthesis from sucrose and glucan synthesis was assumed to represent fructan formation by fructosyltransferase.

Collection of saliva. Samples of whole unstimulated saliva were collected in beakers chilled in ice. The saliva was clarified by centrifugation at 10,000 × *g* for 10 min and frozen until it was used. In some experiments whole saliva samples were absorbed with 10^{10} *S. mutans* JBP or *S. sobrinus* 6715 cells per ml with continuous mixing at room temperature. The streptococcal cells were removed by centrifugation, and the saliva samples were stored frozen until use. Prior to absorption, the saliva agglutinated strains JBP and 6715 cells with titers of 1:64 and 1:2, respectively. After absorption with *S. mutans* JBP, the saliva did not agglutinate cells of either organism. However, saliva absorbed with *S. sobrinus* 6715 cells still agglutinated *S. mutans* JBP cells to a titer of 1:16, although it no longer agglutinated strain 6715 cells.

Submandibular saliva was obtained by using a custom-fitted collector (1). In some experiments we used fractions of submandibular saliva obtained by chromatography on columns of trisacryl GF-2000 (LKB, Bromma, Sweden) which were 1.6 cm in diameter by 33 cm long. The saliva (3.0 ml) was predialyzed against 0.1 M NH_4HCO_3 buffer (pH 7.9) containing 0.5% chloroform, and the column was eluted with this buffer; 3.7-ml fractions were collected and stored frozen until use.

Preparation of experimental pellicles. Experimental pellicles were prepared by exposing 5-mg samples of calcium phosphate beads (spheroidal HA; BDH Chemicals, Gallard-Schlesinger Chemical Corp., Carle Place, N.Y.) to 125-μl portions of either GTF, saliva-GTF mixtures, saliva-saline mixtures, or saliva fractions for 1 h (11). After washing, the HA beads were treated for 30 min with 2 mg of bovine albumin (Sigma Chemical Co., St. Louis, Mo.) per ml in 0.05 M KCl containing 1 mM Ca^{2+} and 1 mM phosphate (pH 6.0) (buffered KCl) to block any uncoated regions of the HA (12). The beads were then washed three times with buffered KCl and incubated with 6.25 × 10^6 [^{3}H]thymidine-labeled streptococcal cells suspended in 125 μl of buffered KCl containing 2 mg of albumin per ml. After 1 h of incubation, the beads were washed three times with buffered KCl, and the number of streptococcal cells which attached to the beads was determined by direct scintillation counting. All values were corrected for quench due to the HA beads. Statistical analyses were performed by using Student's *t* test.

In nine separate experiments, the specific activities of washed strain JBP cells varied between 280 and 1,050 cpm per 10^5 cells. For strain 6715, the specific activities varied from 300 to 1,160 cpm per 10^5 cells. The counts associated with experimental pellicles after bacterial attachment were usually between 500 and 2,000 cpm above the background level of 20 to 25 cpm for strain JBP. For strain 6715, which attached more poorly in most instances, these values varied between 35 and 2,200 cpm above the background level.

RESULTS

Adsorption of *S. mutans* JBP and *S. sobrinus* 6715 to pellicles prepared from saliva-GTF mixtures. In our initial experiments we used a GTF preparation derived from *S. mutans* JBP which formed 85 μg of glucan per h per ml from [^{14}C]glucose-labeled sucrose; this preparation contained 5.4 μg of protein per ml and had a low level of fructosyltransferase activity (Table 1). Heating the enzyme at 60°C for 30 min or at 80°C for 15 min resulted in a complete loss of both GTF and fructosyltransferase activities (Table 1). Samples of unheated clarified whole saliva also had detectable levels of GTF activity (Table 1). Heating the saliva reduced GTF activity but did not completely eliminate it; thus, salivary components may help to stabilize GTF. No GTF activity was detected in submandibular saliva. Mixtures of *S. mutans* GTF and saliva had an activity roughly proportional to the quantity of GTF added (Table 1).

Experimental pellicles formed by exposing HA beads to the GTF preparation alone or to saliva-GTF mixtures exhibited detectable GTF activity (Table 2). This confirmed the observation of Rolla et al. (25, 26) that GTF adsorbs to HA surfaces and is active in the adsorbed state. However, the activity of GTF in pellicles prepared from saliva-saline mixtures was below the level of detection.

The adsorptive behavior of *S. sobrinus* 6715 to experimental pellicles was strikingly different from that of *S. mutans* JBP. *S. mutans* JBP cells adsorbed in much higher numbers to pellicles formed from whole or submandibular saliva than to buffer-treated HA, while *S. sobrinus* cells attached poorly

TABLE 1. GTF activities of GTF preparations from *S. mutans* JBP and of saliva

Sample	GTF activity (μg of glucan per h per ml)
GTF[a]	85.0 ± 2.4
GTF + buffered KCl (1:1)	40.0 ± 2.0
GTF + buffered KCl (1:1) (60°C, 30 min)	<1.0
GTF + buffered KCl (1:1) (80°C, 15 min)	<1.0
Unheated whole saliva	6.0 ± 0.6
Heated whole saliva (60°C, 30 min)	4.0 ± 0.3
Heated whole saliva (80°C, 15 min)	2.0 ± 0.2
Submandibular saliva	<1.0
Unheated whole saliva + GTF (1:1)	50.0 ± 4.2
Unheated whole saliva + GTF (4:1)	13.0 ± 1.5

[a] The GTF preparation from *S. mutans* JBP contained 5.4 μg of protein per ml and assayed 88% GTF with [^{14}C]glucose-labeled sucrose and 12% fructosyltransferase with [U-^{14}C]sucrose.

to both saliva and buffer-treated HA (Table 3). Pellicles formed from GTF-supplemented saliva did not promote adsorption of either organism to a greater extent than pellicles formed from whole or submandibular saliva alone. The inability of GTF supplementation to enhance adsorption did not appear to be attributable to saturation of the pellicles with streptococcal cells. The relatively low concentrations of streptococci used in the assays (6.25×10^6 cells per 125 μl) are well below the concentrations required for saturation (4). Also, under the conditions used, we calculated that less than 1% of the surface area of the HA beads became occupied by adsorbed streptococci at equilibrium. In addition, only 13% of the available streptococci became attached to pellicles formed from saliva or saliva-GTF mixtures.

Both organisms adsorbed in higher numbers to pellicles formed from the GTF preparation alone than to buffer-treated-HA (Table 3). However, the number of *S. mutans* JBP cells which attached to pellicles formed from GTF alone was only one-half the number which attached to pellicles prepared from whole or submandibular saliva. Exposure of pellicles formed from the GTF preparation to sucrose to permit in situ glucan synthesis markedly enhanced adsorption of *S. sobrinus* 6715 ($P < 0.001$), but this treatment had little or no effect on *S. mutans* JBP. Exposure of pellicles formed from saliva-GTF mixtures to sucrose also tended to promote adsorption of *S. sobrinus* 6715 ($P < 0.05$) but not *S. mutans* JBP (Table 3). Collectively, these observations suggest that *S. mutans* JBP cells contain an adhesin which binds to components present in whole and submandibular saliva. *S. sobrinus* 6715 cells evidently lack such a salivary adhesin, but they can bind effectively to components synthesized from sucrose by the adsorbed GTF preparation.

Both *S. mutans* JBP and *S. sobrinus* 6715 exhibited cell-associated GTF activity; these activities were 84 and 46 μg of glucan synthesized per h per 10^8 streptococcal cells, respectively. These assays were performed in the presence of 0.15% NaF to inhibit glycolysis and glycogen synthesis. Thus, the lack of an effect from sucrose exposure of the pellicles on *S. mutans* JBP was not due to the absence of cell-associated GTF.

To determine whether the simultaneous synthesis of glucan from sucrose influenced attachment of *S. mutans* JBP, the ability of this organism to attach to pellicles was tested in the presence and absence of 0.5% sucrose. In the presence of sucrose $64.0 \times 10^4 \pm 7.0 \times 10^4$ and $81.0 \times 10^4 \pm 4.0 \times 10^4$ streptococcal cells attached to pellicles formed from saliva-saline and saliva-GTF mixtures, respectively. In the absence of sucrose, these values were $88.0 \times 10^4 \pm 8.0 \times 10^4$ and $92.0 \times 10^4 \pm 1 \times 10^4$ cells, respectively. Also, no differences were noted when pellicles were prepared from saliva which had been supplemented with heat-inactivated GTF. The apparent inhibition of adsorption of *S. mutans* by sucrose has been observed previously with *S. sobrinus* 6715 (6).

Effect of GTF from different sources on the adsorption of *S. mutans* JBP and *S. sobrinus* 6715 to pellicles. To determine whether GTFs derived from different species behaved similarly, enzyme preparations were obtained from *S. mutans* JBP, *S. sobrinus* 6715, and *Streptococcus sanguis* FC-1. Each of these preparations had higher GTF activities than the preparations used in the experiments reported in Tables 1 and 3 (Table 4). *S. mutans* JBP cells did not adsorb well to HA which had been exposed to any of these GTF preparations, although the organism did attach well to pellicles prepared from saliva-saline or saliva-GTF mixtures (Table 4). Also, exposure of these pellicles to sucrose to permit glucan synthesis did not have a marked effect on *S. mutans* JBP attachment. In sharp contrast, *S. sobrinus* 6715 cells adsorbed in much higher numbers to HA which had been treated with these GTF preparations than to buffer-treated HA (Table 4), even in the absence of sucrose. However, pellicles prepared from saliva-GTF mixtures were not more effective in promoting attachment of the organism than buffer-treated HA (Table 4). Exposure of pellicles prepared from the GTF alone or from saliva-GTF mixtures to sucrose further enhanced attachment of *S. sobrinus* 6715. Since pellicles formed from saliva-GTF mixtures contained detectable GTF activity (Table 2), their inability to promote adsorption of strain 6715 cells in the absence of sucrose exposure suggests that it is glucan, rather than GTF, that serves as the pellicle receptor for this organism. The greater attachment of strain 6715 cells to HA beads exposed to these GTF preparations in the absence of sucrose may reflect a higher degree of glucan contamination from the Sephadex affinity adsorbent than was present in the preparation used in the experiments reported in Table 3.

Adsorption of *S. mutans* JBP and *S. sobrinus* 6715 to pellicles prepared from dextran and saliva-dextran mixtures. *S. mutans* JBP cells did not adsorb better to HA which had been pretreated with dextran 2000 (molecular weight, 2×10^6) than to buffer-treated HA (Table 5). The organism attached comparably well to pellicles prepared from saliva or saliva-dextran mixtures (Table 5). In contrast, *S. sobrinus* 6715 cells attached in higher numbers to HA which had been pretreated with dextran than to pellicles formed from saliva or saliva-dextran mixtures ($P < 0.01$) (Table 5). These

TABLE 2. GTF activities of experimental pellicles

Pellicle prepared from:	Pellicle-associated GTF activity (μg of glucan per h per 5 mg of HA)
Albumin (2 mg/ml)	<0.1
GTF[a]	2.7 ± 0.8
GTF (100°C, 15 min)	<0.1
Unheated saliva + saline (1:1)	<0.1
Unheated saliva + GTF (1:1)	2.2 ± 1.3

[a] GTF from *S. mutans* JBP. For the activity of the preparation used, see Table 1, footnote *a*.

TABLE 3. Adsorption of *S. mutans* JBP and *S. sobrinus* 6715 to pellicles prepared from saliva-GTF mixtures

Pellicle prepared from:	Pellicles exposed to 5% sucrose for 30 min	*S. mutans* JBP		*S. sobrinus* 6715	
		No. of streptococci adsorbed (10^4)[a]	% Relative to whole saliva-saline	No. of streptococci adsorbed (10^4)[a]	% Relative to whole saliva-saline
Buffered KCl	−	6.0 ± 1.3[b]	7	1.2 ± 0.1[b]	120
Whole saliva + saline (1:1)	−	81.0 ± 2.0	100	1.0 ± 0.1	100
Whole saliva + GTF (1:1)[c]	−	83.0 ± 6.0	102	1.3 ± 0.1	130
Submandibular saliva + saline (1:1)	−	86.0 ± 4.0	106	1.1 ± 0.1	110
Submandibular saliva + GTF (1:1)	−	80.0 ± 12.0	99	1.4 ± 0.2	140
GTF	−	37.0 ± 7.0	46	2.5 ± 0.7	250
Buffered KCl	+	10.0 ± 1.0	12	1.6 ± 0.3	160
Whole saliva + saline (1:1)	+	87.0 ± 6.0	107	1.6 ± 0.1	160
Whole saliva + GTF (1:1)	+	90.0 ± 6.0	111	3.4 ± 0.3	340
Submandibular saliva + saline (1:1)	+	86.0 ± 8.0	106	1.6 ± 0.1	160
Submandibular saliva + GTF (1:1)	+	82.0 ± 10.0	101	2.4 ± 0.1	200
GTF	+	42.0 ± 2.0	52	17.0 ± 0.4	1,700

[a] The reaction mixtures (125 μl) contained 5 mg of spheroidal HA beads and 6.25 × 10^6 ^{3}H-labeled streptococcal cells suspended in buffered KCl.
[b] Mean ± standard error.
[c] GTF from *S. mutans* JBP. For the activity of the preparation used, see Table 1, footnote *a*.

observations confirm that the presence of glucan on HA surfaces promotes the adsorption of *S. sobrinus* 6715 cells but not cells of *S. mutans* JBP. Our data also suggest that in the presence of saliva, dextran either does not adsorb effectively to HA or becomes occluded or destroyed by salivary components or enzymes.

Adsorption of *S. mutans* JBP and *S. sobrinus* 6715 to pellicles prepared from untreated and streptococcus-absorbed saliva samples. *S. mutans* JBP attached in lower numbers ($P < 0.01$) to pellicles formed from saliva which had been preabsorbed with strain JBP cells (Table 6). Preabsorption with strain 6715 cells had less effect; significantly ($P < 0.05$) higher numbers of *S. mutans* JBP cells attached to pellicles prepared from saliva which had been preabsorbed with strain 6715 cells than to pellicles preabsorbed with strain JBP cells (Table 6). In contrast, absorption of saliva with either strain JBP cells or strain 6715 cells did not affect the number of *S. sobrinus* 6715 cells which attached (Table 6). These observations are consistent with the notion that *S. mutans* JBP cells possess an adhesin which reacts with salivary components, whereas *S. sobrinus* 6715 cells do not.

Adsorption of *S. mutans* JBP and *S. sobrinus* 6715 to pellicles prepared from fractions of submandibular saliva. Pellicles prepared from the high-molecular-weight mucin fraction of submandibular saliva were as effective as pellicles prepared from unfractionated saliva in promoting *S. mutans* JBP attachment (Fig. 1). None of the other saliva fractions significantly enhanced attachment of strain JBP cells above

TABLE 4. Effect of GTF preparations from different sources on adsorption of *S. mutans* JBP and *S. sobrinus* 6715 to experimental pellicles

Pellicle prepared from[a]:	Pellicles exposed to 5% sucrose for 30 min	*S. mutans* JBP		*S. sobrinus* 6715	
		No. of streptococci adsorbed (10^4)	% Relative to saliva-saline	No. of streptococci adsorbed (10^4)	% Relative to saliva-saline
Buffered KCl	−	6.4 ± 0.8[b]	10	3.2 ± 0.1[b]	128
GTF from strain JBP	−	4.4 ± 1.0	7	57.0 ± 1.0	2,190
GTF from strain 6715	−	5.5 ± 0.5	9	52.0 ± 7.0	2,000
GTF from strain FC-1	−	5.5 ± 0.8	9	38.0 ± 3.5	1,460
Saliva + saline (1:1)	−	64.5 ± 1.5	100	2.6 ± 4.0	100
Saliva + GTF from strain JBP (1:1)	−	76.0 ± 8.0	118	3.3 ± 0.3	127
Saliva + GTF from strain 6715 (1:1)	−	93.2 ± 2.8	144	3.1 ± 0.3	119
Saliva + GTF from strain FC-1 (1:1)	−	63.1 ± 7.0	98	2.7 ± 0.2	104
Buffered KCl	+	10.4 ± 2.6	16	5.0 ± 1.2	192
GTF from strain JBP	+	15.0 ± 2.0	23	33.0 ± 0.2	1,270
GTF from strain 6715	+	8.8 ± 0.2	14	123.0 ± 17.0	4,730
GTF from strain FC-1	+	4.3 ± 0.3	7	89.0 ± 8.0	3,420
Saliva + saline (1:1)	+	62.0 ± 0.2	96	3.4 ± 0.2	130
Saliva + GTF from strain JBP (1:1)	+	87.0 ± 0.2	135	14.0 ± 1.0	538
Saliva + GTF from strain 6715 (1:1)	+	69.0 ± 5.0	107	135.0 ± 4.5	5,190
Saliva + GTF from strain FC-1 (1:1)	+	60.0 ± 3.0	93	16.0 ± 1.5	615

[a] GTF from *S. mutans* JBP contained 5.4 μg of protein per ml and synthesized 274 μg of glucan per h per ml. GTF from *S. sobrinus* 6715 contained 18.7 μg of protein per ml and synthesized 1,096 μg of glucan per h per ml. GTF from *S. sanguis* FC-1 contained 3.8 μg of protein per ml and synthesized 390 μg of glucan per h per ml.
[b] Mean ± standard error.

TABLE 5. Effect of dextran on adsorption of *S. mutans* JBP and *S. sobrinus* 6715 to pellicles

Pellicle prepared from:	*S. mutans* JBP		*S. sobrinus* 6715	
	No. of cells adsorbed (10^4)	Relative %	No. of cells adsorbed (10^4)	Relative %
Buffered KCl	8.0 ± 1.0[a]	10	1.9 ± 0.2[a]	126
Saliva[b]	80.0 ± 7.0	100	1.5 ± 0.1	100
Saliva + H_2O (4:1)	74.0 ± 7.0	93	1.3 ± 0.1	87
Saliva + dextran (10 mg/ml) (4:1)[c]	97.0 ± 9.0	121	1.4 ± 0.3	93
Saliva + dextran (0.5 mg/ml) (4:1)	93.0 ± 8.0	116	1.5 ± 0.1	100
H_2O + dextran (10 mg/ml) (4:1)	7.0 ± 1.0	9	8.7 ± 0.3	580
H_2O + dextran (0.5 mg/ml) (4:1)	8.0 ± 1.0	10	10.1 ± 1.9	673

[a] Mean ± standard error.
[b] Unheated clarified whole saliva.
[c] Dextran 2000 (Pharmacia) was used.

the level of attachment to buffer-treated HA. Also, none of the saliva fractions promoted attachment of *S. sobrinus* 6715 cells (Fig. 1).

Adsorption of additional strains of *S. mutans* and *S. sobrinus* to pellicles prepared from saliva, dextran, or buffer. Adsorption of five strains of *S. mutans* (strains JBP, MT3, S3, P2, and AT6) and five strains of *S. sobrinus* (strains 6715, OMZ-176, MB2R, 14H, and SL-1) was examined by using HA which had been treated with clarified whole saliva or with 5 mg of dextran 2000 per ml to determine whether the differences observed were representative of these species. All five strains of *S. mutans* adsorbed in higher numbers to saliva-treated HA than to buffer-treated HA (mean ratio, 2.04 ± 0.55), but none of the five strains of *S. sobrinus* studied did (mean ratio, 0.76 ± 0.11). Also, none of the *S. mutans* strains adsorbed in higher numbers to dextran-treated HA than to buffer-treated HA (mean ratio, 0.75 ± 0.15), whereas all five strains of *S. sobrinus* showed greatly enhanced adsorption to dextran-treated HA (mean ratio, 8.5 ± 3.5).

DISCUSSION

Our data revealed fundamental differences in the way that strains of *S. mutans* and *S. sobrinus* attach to pellicles similar to those on teeth. Strains of *S. mutans* attached in higher numbers to pellicles prepared from whole or submandibular saliva than strains of *S. sobrinus* did. In fact, *S. sobrinus* strains did not attach better to saliva-treated HA than to buffer-treated HA when albumin blocking was used.

TABLE 6. Adsorption of *S. mutans* JBP and *S. sobrinus* 6715 to pellicles formed from untreated and streptococcus-absorbed saliva samples

Pellicles prepared from:	*S. mutans* JBP		*S. sobrinus* 6715	
	No. of streptococci adsorbed (10^4)	Relative %[a]	No. of streptococci adsorbed (10^4)	Relative %
Untreated saliva	77.3 ± 1.9[b]	100	8.0 ± 0.21[b]	100
Strain JBP-absorbed saliva	27.1 ± 0.35	35	9.1 ± 0.01	114
Strain 6715-absorbed saliva	59.4 ± 0.60	77	8.1 ± 0.04	109
Buffered KCl	17.8 ± 0.46	14	7.0 ± 0.11	88

[a] For untreated saliva versus strain JBP-absorbed saliva, $P < 0.01$; for strain JBP-absorbed saliva versus strain 6715-absorbed saliva, $P < 0.05$.
[b] Mean ± standard error.

The failure of salivary components to promote the adsorption of *S. sobrinus* strains is consistent with the recent report that attachment of *S. sobrinus* 6715 to salivary pellicles is nonsaturable and involves nonspecific interactions (31).

That *S. sobrinus* 6715 lacks an adhesin which is reactive with salivary components was also supported by the observation that absorption of saliva with strain 6715 cells prior to

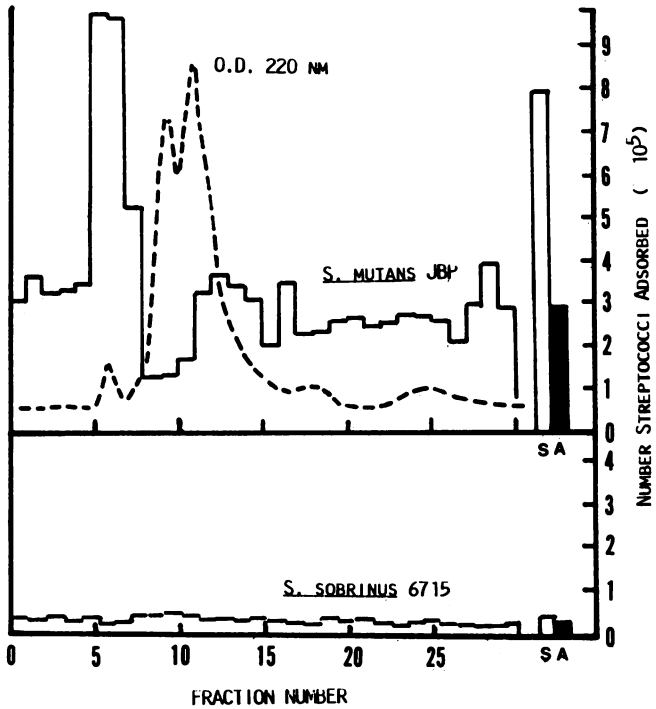

FIG. 1. Adsorption of *S. mutans* JBP and *S. sobrinus* 6715 to experimental pellicles formed from submandibular saliva (S), from albumin (A), and from fractions of submandibular saliva obtained by chromatography on columns of trisacryl GF-2000. The reaction mixtures and assay conditions used were the same as those used in other experiments. The dashed line shows the elution profile of the column, as determined by optical density at 220 nm (O.D. 220 NM). Fractions 6 and 7 of submandibular saliva gave pellicles that promoted adsorption of *S. mutans* JBP significantly above the adsorption to albumin-treated HA. Neither submandibular saliva nor its fractions promoted adsorption of *S. sobrinus* 6715 above the level of adsorption to albumin-treated HA.

its use in pellicle formation had little effect upon the numbers of either *S. sobrinus* 6715 or *S. mutans* JBP cells which subsequently attached. However, prior absorption with *S. mutans* JBP cells removed or destroyed the receptor for this organism, but this treatment did not affect attachment of *S. sobrinus* 6715.

Whole saliva contains salivary components and bacterial products, and either could be responsible for promoting attachment of *S. mutans* JBP to pellicles. However, the finding that pellicles formed from submandibular saliva were equally or more effective in promoting attachment indicates that this organism possesses an adhesin which interacts with a component(s) of saliva. Similar results have also been obtained with samples of parotid saliva (data not shown). When submandibular saliva was fractionated on trisacryl GF-2000 columns, only high-molecular-weight components present in the void volume strongly promoted attachment. This fraction contained all of the blood group-reactive mucins of the saliva, which suggests that these molecules may serve as receptors for the *S. mutans* adhesin. The results which we obtained are consistent with our previous report that certain blood group-reactive components present in complex culture media can be found on the surfaces of strains of *S. mutans*, but not on cells of *S. sobrinus* strains 6715 and Bob 1 (13).

As previously noted by Rolla and co-workers (25, 26), GTF activity is detectable in whole saliva and in experimental pellicles formed either from GTF-supplemented saliva or from GTF preparations alone. We did not detect GTF activity in pellicles formed from unsupplemented whole saliva, presumably because any GTF activity present was below the detection limit of the assay used. However, neither *S. mutans* JBP nor *S. sobrinus* 6715 attached better to pellicles which contained demonstrable GTF activity than to pellicles formed from saliva-saline mixtures or from submandibular saliva. Thus, GTF per se does not appear to constitute an important pellicle receptor for these streptococci. However, when pellicles were exposed to sucrose to permit glucan synthesis, the pellicles which contained high GTF activity enhanced attachment of *S. sobrinus* 6715 but not attachment of *S. mutans* JBP. This suggests that *S. sobrinus* 6715 cells possess an adhesin which binds to the glucan synthesized from sucrose. This possibility was supported by the observation that *S. sobrinus* 6715 cells adsorb in high numbers to dextran-treated HA. However, dextran did not promote attachment of *S. mutans* JBP, and therefore this organism lacks a functional dextran-binding adhesin. Since washed cells of both species contained high levels of GTF activity, it seems unlikely that cell-associated GTF is the glucan-binding adhesin responsible, even though this enzyme is known to bind to dextran (28). These observations are consistent with the report of Inoue and co-workers (19) that serotype d and g mutans streptococci (*S. sobrinus*) agglutinate with dextran, while serotypes c, e, and f strains (*S. mutans*) do not. It is interesting that these investigators also observed that *S. sobrinus* strains attached in lower numbers to saliva-treated HA than strains of *S. mutans* did (19). Our findings are also consistent with the report of Tinanoff et al. (32) that strains of *S. mutans* form accumulations on pieces of enamel in in vitro cultures in the absence of sucrose, but *S. sobrinus* 6715 does not.

While dextran treatment of HA was effective in promoting *S. sobrinus* 6715 attachment, HA treatment with saliva-dextran mixtures was not. Thus, either salivary components interfere with the adsorption of dextran to HA, or salivary components mask or degrade adsorbed dextran molecules. Rolla (23) has noted that salivary components can inhibit the binding of dextran to HA surfaces (23). Pretreatment of HA with GTF preparations derived from *S. mutans* JBP, *S. sobrinus* 6715, and *S. sanguis* FC-1 also enhanced attachment of *S. sobrinus* 6715 cells, presumably because these preparations contained some glucan derived either from the organisms or from the Sephadex beads used for isolating the GTF.

The differences observed between strains JBP and 6715 appear to be characteristic of the species *S. mutans* and *S. sobrinus*. All five strains of *S. mutans* studied exhibited enhanced attachment to saliva-treated HA compared with buffer-treated HA, whereas none of the *S. sobrinus* strains tested did this. We have also observed that *S. mutans* strains LM7 (serotype e) and OMZ-175 (serotype f) adsorb better to salivary pellicles than to buffer-treated HA (data not shown), and thus the observations which have been made appear to be applicable to all three serotypes of *S. mutans*. Likewise, the *S. sobrinus* strains studied included serotypes d and g, and all strains attached in much higher numbers to dextran-treated HA than to buffer-treated HA.

It is somewhat surprising that the marked differences observed in the attachment of *S. mutans* and *S. sobrinus* strains to pellicles were not clearly noted previously. The albumin-blocking procedure (12) used in the present study significantly contributed to recognition of these differences, because it enabled us to distinguish between bacterial attachment to adsorbed pellicle components and attachment to any uncoated areas of the HA surface. In effect, albumin blocking established the weak interaction of these streptococci with albumin as a base line so that higher-affinity interactions between the organisms and other adsorbed molecules could be discerned.

The fundamental differences observed in the attachment of *S. mutans* and *S. sobrinus* to pellicles reinforces the need to consider these organisms as separate and distinct species, as has been suggested previously (7). In this regard, although *S. mutans* is much more prevalent in humans than *S. sobrinus* (16, 21, 27), strains of the latter organism have often been used in animal experiments and in attempts to develop protective vaccines for dental caries. One reason for this is that strain 6715 has maintained a high level of virulence for animals over many years, whereas strains of *S. mutans* frequently lose virulence-associated properties (34). It is interesting to speculate that this may be because *S. mutans* strains lose their salivary adhesin when they are repeatedly cultured in the absence of saliva, whereas strains of *S. sobrinus* maintain their glucan-binding adhesin because of the wide distribution of traces of glucans and sucrose in natural culture media.

Since strains of *S. mutans* and *S. sobrinus* appear to attach to different receptors in pellicles, it seems likely that their host ranges and patterns of colonization may also exhibit differences. One would expect glucans to be present on the teeth of a variety of host species who consume sucrose-containing foods, and thus one would suspect that *S. sobrinus* would display a wider range of hosts which it can colonize under natural conditions than *S. mutans*. This may in fact be partially responsible for the high frequency with which *S. sobrinus* strains have been studied in rats and hamsters. In any case, it seems clear that investigators seeking to control or prevent dental caries by attempting to interfere with the colonization of the mutans group of streptococci should be cognizant of the fundamental differences between the ways in which *S. mutans* and *S. sobrinus* attach to surfaces similar to tooth surfaces.

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